Microindentation in the study of bone properties for type 1 Diabetes Mellitus

Patients

Running Title: Microindentation in type 1 diabetes

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ABSTRACT

Objective: To analyse the bone quality in type 1 diabetes mellitus (T1DM) women and healthy controls, as well as their relationship with bone mineral density (BMD).

Research design and methods: Cross-sectional study including 45 premenopausal women with T1DM and 21 healthy controls matched by age and BMI. Clinical parameters, BMD and bone tissue mechanical properties through bone material strength index (BMSi) were evaluated in both groups by using microindentation. In T1DM patients, glycosylated hemoglobin (HbA_{1c}), the number of hypoglycaemias and the chronic complications state were also analysed.

Results: No differences between T1DM patients and healthy controls were observed in BMSi or in BMD. Among T1DM patients, mean HbA_{1c} was $7.52 \% \pm 1.0$ and mean time since diagnosis was 22.6 ± 12.2 years. 8 patients (17.7 %) met the Metabolic Syndrome (MetS) criteria, and in 12 patients (26.7 %) microvascular complications were present. A trend towards a lower BMSi was observed in parallel to an increase of the number of MetS criteria and in patients with microangiopathy.

Conclusions: T1DM premenopausal patients show bone tissue properties comparable to controls. Microangiopathy and MetS seem to affect negatively to BMSi.

INTRODUCTION

Being affected of diabetes mellitus (DM) involves an increased risk of hip fracture, more significant in patients with type 1 diabetes mellitus (T1DM) [1][2][3] and more pronounced that what could be explained from decreased BMD in this group [4], so it suggests that the presence of DM plays a role on bone quality independent of BMD [5][6]. Glycaemic control and DM- related complications have been linked to this risk of bone fractures, but patients with T1DM, moreover, can present some predisposing factors that would raise this risk, such as a lower peak bone mass and lower BMD [7][8], increased risk of falls due to hypoglycaemia [9], and common presence of sensory neuropathy and retinopathy [4].

Unlike the exposed for the T1DM, patients with type 2 diabetes mellitus (T2DM) presented an increased risk of hip fracture despite having a higher BMD [4]. Nonetheless, in the last years it has been described a trend in T1DM patients to keep a similar type 2 diabetes mellitus (T2DM) phenotype in terms of overweight or obesity, metabolic syndrome (MetS), insulin resistance and other cardiovascular risk factors [10], but has not been directly analysed the impact on bone tissue of these alterations.

In regard of markers of bone turnover, in diabetes seems that exists abnormalities in bone formation phase reflected in lower osteocalcin levels, both in patients with T1DM and T2DM [4][5], and elevated levels of sclerostin, that only have been reported in T2DM [4]. For results for bone resorption markers most studies have reported normal levels [4]. It has been proposed that these abnormalities on bone formation result in impaired bone quality or bone microarchitecture not assessable by DXA [5], which has been assessed with quantitative computed tomography and others related imaging techniques, histomorphometric studies and more recently with microindentation [4][11].

Microindentation directly measures bone tissue quality assessing the resistance to open microcracks in cortical bone after a controlled impact. Bone microindentation correlates with a fracture propensity through a unitless variable, bone material strength index (BMSi) [11]. Recent evidence has displayed that BMSi was lower in subjects with T2DM compared to controls [12] but, for now, no data available about BMSi to investigate bone quality in T1DM.

For all the above and with the hypothesis that patients with T1DM have deterioration of bone tissue quality measured by microindentation, we have developed a cross-sectional study whose main objective is to analyse the bone tissue quality by microindentation in premenopausal T1DM women and healthy controls, and to establish their relationship with BMD. Secondary aims are to assess the possible impact of glycaemic control, presence of microangiopathy or presence of MetS criteria, on the bone health in this specific population group.

RESEARCH DESIGN AND METHODS

Study population

In reference to estimation of the size of the sample, accepting an alpha risk of 0.05 and a beta risk of 0.2 in a bilateral contrast, we calculated that 35 subjects in each of the groups were required to detect in the BMSi an equal or greater difference to 5 units. It was assumed that the common standard deviation was 7 units.

Finally, we performed a cross-sectional study including 45 premenopausal women with T1DM and 21 healthy subjects as a control group, matched by age and body mass index. It was conducted between November 2013 and May 2017.

The inclusion criteria were women over 18 years with T1DM at least with one year of duration. Controls were age and body mass index (BMI) matched subjects recruited from the general population during the same period.

Exclusion criteria were women with osteoporosis or previous pathologic fractures, patients with previous or current chronic treatment with high-dose corticosteroids or a thiazolidinedione, presence of diseases that predispose to the development of osteoporosis (hypogonadism, panhypopituitarism or Cushing's syndrome), and patients who did not consent to participate in the study.

The study was approved by “Comitè d'Ètica de la Investigació amb Medicaments (CEIm)” ethical committee, which was performed in accordance with the Declaration of Helsinki. Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

Clinical evaluation and laboratory data

We noted toxic habits (smoking and alcohol intake) and history of hypertension or dyslipidaemia. Anthropometric variables (weight, height, blood pressure) were collected using standardized methods.

In T1DM patients, the presence of complications in accordance with the American Diabetes Association criteria [13] was assessed as follows: cardiovascular disease, nephropathy and neuropathy were evaluated by two expert diabetologists (JJC, AG), and retinopathy was assessed by an ophthalmologist after pharmacological mydriasis. Microangiopathy was defined for the presence of retinopathy, neuropathy and/or nephropathy. Also related variables, such as T1DM duration, treatment, insulin doses and presence of hypoglycaemia (defined as capillary blood glucose less than 70 mg/dl [13]), were collected.

NCEP-ATP III modified criteria were used to define metabolic syndrome (MetS) [14] and insulin sensitivity was assessed with the estimated glucose disposal rate (eGDR) [15].

A blood analysis was performed in all subjects, including basal glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, calcidiol, calcium, phosphate, iPTH and bone turnover markers. In T1DM patients, glycated hemoglobin and albumin/creatinine ratio in sporadic urine was also determined.

Bone mineral density and bone material strength index

BMD was measured in the lumbar spine, femoral neck and total femur in all subjects by dual energy x-ray absorptiometry (DXA) using a Hologic QDR 4500 SR Bone Densitometer (Hologic, Inc., Bedford, MA, USA).

BMSi was obtained by microindentation technique by inserting a probe assembly through the skin covering the tibia and, after displacing periosteum, applying 20 indentation cycles at 2 Hz each with a maximum force of 11 N, using the Osteoprobe device (Active Life Scientific, Santa Barbara, CA, USA), as previously reported [11][16].

Statistical analysis

Student t test was used to evaluate the difference between means, and the Chi-squared method was employed to assess the association between categorical variables. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows, v.12.0, Chicago, IL).

RESULTS

Participant characteristics

Among T1DM patients, mean HbA_{1c} was 7.52 % $\pm$ 1.0 and the mean time since diagnosis was 22.6 $\pm$ 12.2 years. 8 patients (17.7 %) met the MetS criteria, and in 12 patients (26.7 %) microvascular complications were present.

General characteristics, clinical and biochemical features of participants are shown in Table 1.

Bone characteristics: BMSi, BMD and bone turnover markers

No differences in the in BMSi were observed between T1D and control group (79.9 $\pm$ 8.5 in T1DM vs 80.3 $\pm$ 7.1 in controls, $P = 0.825$). Either difference in the BMD was not found. Densitometric and microindentation data of patients with T1DM and control subjects are shown in Table 2. Bone turnover markers in patients with T1DM grouped in base the cohort BMSi mean are shown in Table 3, no data of bone turnover markers of control subjects were available. Only an increase in P1NP in those T1D with BMSi less than 80 achieved the statistical significance (56.20 $\pm$ 28.50 in T1DM with BMSi less than 80 vs 33.90 $\pm$ 8.26 in T1DM with BMSi greater than 80, $P = 0.047$).

Impact of glycaemic control, microangiopathy and metabolic syndrome

Any relationship between fasting glucose either HbA_{1c} and bone turnover markers are found (data not shown). Though no significant differences were found, a trend towards to lower BMSi was observed in parallel to an increase of the number of MetS criteria (79.2 $\pm$ 8,5 vs 75.1 $\pm$ 7.9, $P = 0.273$, 1 vs 3 or more MetS criteria) and in patients with microangiopathy (78.3 $\pm$ 10.8 vs 80.5 $\pm$ 7.6, $P = 0.447$, with or without microangiopathy, respectively).

DISCUSSION

Several population-based studies have shown T1DM patients have an increased risk of fracture compared to the general population [1][2][4]. Indeed, it is necessary to emphasize the work of Janghorbani, et al. in that described an increased risk of hip fracture in both type 1 and type 2 diabetes in the 22-year follow-up “The Nurses’ Health Study”. Unlike these studies, our work was performed in a specific subgroup of T1DM patients, concretely premenopausal women without a history of osteoporosis either fractures, to evaluate bone quality with microindentation in a cross-sectional study.

No differences were found in the BMSi in T1DM patients respect the control group, but given that this is the first study that has evaluated the BMSi in T1DM, have not previous studies to compare the results. Either no difference was found in BMD between the study groups. Despite that few previous studies showed that patients with T1DM usually presented a lower peak bone mass and lower BMD [7][8], until very recently large, long-term natural history studies evaluating changes in BMD over time were lacking [4]. In September 2018, Hamilton, et al. reported a longitudinal observational study about 20 males and 28 females with T1DM, with a median diabetes duration 14.6 years, which were follow-up a mean of 10.3 years to determine temporal BMD changes and reflected changes in bone turnover markers [17]. In that study, females with T1DM exhibited no change in BMD or T-score at any site after adjustment for age, BMI and postmenopausal status (50% at the end of follow-up), the fact that supports our findings.

Factors that can contribute in these results are hypothesized below. First, the degree of metabolic control in the present study was good, the percentage of T1DM-related complications was low, and had a limited number of hypoglycaemia, all of them factors that have been related to impaired bone health and increase fracture risk [4]. Second, in line with Weber, et al. proposes [4], some of the principal studies of bone health in

T1DM include patients with diagnoses before 1993, when intensive insulin therapy was established how standard of care for the treatment of T1DM as a result of the publication of the Diabetes Control and Complication Trial [18]. Before this, patients may have been exposed to poor glycaemic control with the detriment of bone health as a consequence, compared with patients included in recent studies like ours. Finally, other studies had been performed in postmenopausal DM women [5] and it is also possible that estrogenic deprivation following menopause affects the bone more intensively in patients with DM than those who do not suffer from it, or have other factors so far unknown to explain this increased risk.

With no impact in BMSi or BMD, it is remarkable that in our T1DM patients with a BMSi lower than the mean presented significant elevation of P1NP respect those with higher BMSi. This finding can be contextualized in line of most previous studies that described alterations on levels of bone formation markers in association with T1DM, while most studies, like ours, reported normal levels of bone resorption markers [4], supporting the idea that in diabetes exists abnormalities in bone formation phase [4][5].

As secondary aims, the possible impact of glycaemic control, presence of microangiopathy and presence of MetS criteria for the bone health were evaluated. In our cohort no statistical difference in BMI or lipid profile were found between T1DM and controls, which is an expected fact in terms that it has been described a trend in T1DM patients to keep a similar T2DM phenotype in terms of overweight or obesity and MetS [10]. Despite that few studies reported an inverse relationship between non-fasting glucose and HbA_{1c} with bone formation markers (P1NP, osteocalcin) and bone resorption markers (CTX) suggesting a negative effect of hyperglycaemia on bone turnover [4], any relationship was found in our study.

Likely due to small sample size, observed data did not reach statistically significant differences in BMSi between patients with and without microangiopathy, although a trend towards a poorer bone quality in patients with a microangiopathic complication seems to be present. This fact, it is not explained by other variables analysed such as age, BMI or HbA_{1c}, although not either found differences in markers of bone turnover. The characteristic proinflammatory state of insulin resistance and MetS may play a role in this pathogenic state. In this sense, a decrease in BMSi in parallel to the increase in the number of metabolic syndrome criteria was also observed, which again does not reach statistical significance.

The main limitation of the study is its small sample size. Moreover, the cross-sectional design does not consider the possible temporal variability that the evaluated parameters may experience, and secondly, that the findings are only associations but do not imply causality. Likewise, another limitation of this work is the absence of data from bone markers for the control group, which limits the comparison of results at that level. Additionally, this study was limited to a very specific group of patients with long-standing T1DM who are young and premenopausal, making not possible to extrapolate the results to all patients affected to T1DM.

In conclusion, this study did not show differences in the BMSi or BMD in premenopausal T1DM patients compared with healthy controls. However, a trend towards a worse BMSi in those with microangiopathy or MetS will have to be confirmed in future studies.

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Author contributions

a) Conception and design

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b) Acquisition of data

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c) Analysis and interpretation of data

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d) Drafting the article

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e) Final approval of the completed article

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Disclosure statement

Authors have nothing to disclose.

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Table 1. General characteristics, clinical and biochemical features of patients with T1DM and controls subjects

Characteristic	T1DM n=45	Control n=21	<i>P value</i>
Age (years $\pm$ SD)	39.3 $\pm$ 10.2	38.4 $\pm$ 9.9	0.729
Smoker, n (%)	15 (34.1%)	6 (31.6%)	0.145
T1DM evolution (years $\pm$ SD)	22.6 $\pm$ 12.2	---	NA
HbA _{1c} (%) $\pm$ SD)	7.5 $\pm$ 1.0	---	NA
Retinopathy, n (%)	12 (26.7%)	---	NA
Neuropathy, n (%)	3 (6.7%)	---	NA
Nephropathy, n(%)	5 (11.1%)	---	NA
Microangiopathy, n (%)	12 (26.7%)	---	NA
Hypoglycemia per month (n $\pm$ SD)	10.3 $\pm$ 14.9	---	NA
eGDR (mg/kg.min $\pm$ SD)	8.9 $\pm$ 2.1	---	NA
Insulin requirements (U/kg weight/day $\pm$ SD)	0.6 $\pm$ 0.2	---	NA
BMI (kg/m ² $\pm$ SD)	25.2 $\pm$ 4.9	24.6 $\pm$ 4.6	0.617
Glucose (mg/dL $\pm$ SD)	166.8 $\pm$ 79.2	82.7 $\pm$ 11.3	<0.001
Cholesterol (mg/dL $\pm$ SD)	182.8 $\pm$ 25.3	197.6 $\pm$ 34.4	0.067
cLDL (mg/dL $\pm$ SD)	99.0 $\pm$ 25.8	111.4 $\pm$ 23.9	0.084
cHDL(mg/dL $\pm$ SD)	72.2 $\pm$ 17.5	75.9 $\pm$ 20.5	0.470
Triglycerides (mg/dL $\pm$ SD)	78.4 $\pm$ 42.4	73.6 $\pm$ 27.1	0.658

SD: standard deviation; BMI: body mass index; n: subject number; T1DM: type 1 diabetes mellitus; eGDR: *estimated glucose disposal rate*; HbA_{1c}: glycosylated hemoglobin; cLDL: low density lipoproteins; cHDL: high density lipoproteins; n.s.: not significant; NA: not applicable.

1 **Table 2. Densitometric and microindentation data of patients with T1DM and**
2 **controls subjects**

Characteristic	T1DM n=45	Control n=21	<i>P value</i>
D vitamin (ng/mL $\pm$ SD)	25.2 $\pm$ 11.8	26.0 $\pm$ 11.4	0.818
Calcium (mg/dL $\pm$ SD)	9.6 $\pm$ 0.4	9.5 $\pm$ 0.3	0.653
Phosphate (mg/dL $\pm$ SD)	3.7 $\pm$ 0.6	3.5 $\pm$ 0.4	0.383
PTH (pg/mL $\pm$ SD)	44.2 $\pm$ 28.1	36.2 $\pm$ 14.6	0.542
BMSi	79.9 $\pm$ 8.5	80.3 $\pm$ 7.1	0.825
BMSi Microangiopathy No microangiopathy	78,2 $\pm$ 10,8 80,5 $\pm$ 7,6	---	0.447
BMSi 1 MetS criteria 2 MetS criteria $\geq$ 3 MetS criteria	79,2 $\pm$ 8,5 84,7 $\pm$ 9,9 75,1 $\pm$ 7,9	---	0.273
DXA femoral neck (g/cm ² $\pm$ SD)	0.783 $\pm$ 0.112	0.789 $\pm$ 0.129	0.854
DXA total femur (g/cm ² $\pm$ SD)	0.872 $\pm$ 0.146	0.857 $\pm$ 0.110	0.713
DXA lumbar column (g/cm ² $\pm$ SD)	0.993 $\pm$ 0.116	1.008 $\pm$ 0.128	0.684

3 SD: standard deviation; n: subject number; T1DM: type 1 diabetes mellitus; BMSi:

4 *Bone Material Strength index; DXA: Dual Energy X-ray Absorptiometry.*

Table 3. Bone metabolism markers of patients with T1DM with BMSi < 80 vs patients with T1DM with BMSi ≥ 80.

Characteristic	BMSi < 80 N =12	BMSi ≥ 80 N = 8	<i>P value</i>
Homocistein (mcmol/L ± SD)	9.401 ± 5.336	8.159 ± 2.99	0.559
P1NP (ng/mL ± SD)	56.202 ± 28.494	33.895 ± 8.260	0.047
BAP (mcg/L ± SD)	13.611 ± 6.396	10.721 ± 4.195	0.238
CTX (ng/mL ± SD)	0.304 ± 0.131	0.225 ± 0.132	0.216
Adiponectin (mcg/mL ± SD)	20.042 ± 10.730	28.162 ± 11.912	0.143
Leptin (ng/mL ± SD)	25.491 ± 22.510	27.537 ± 18.463	0.831

SD: standard deviation; n: subject number; T1DM: type 1 diabetes mellitus, BAP: bone alkaline phosphatase.